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Effects of calcium treatment applied around the root zone on nutrient concentrations and morphological traits of papaya seedlings (*Carica papaya* L. cv. Eksotika II)

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Abstract

A potculture experiment was carried out to study the effect of calcium applied to the root zone of papaya seedlings (*Carica papaya* L. cv. Eksotika II) on nutrient uptake and morphological traits during 2010-11 at Agro technology Unit, University Agriculture Park (TPU), Universiti Putra Malaysia, Serdang, Selangor. Papaya seedlings established in pots fertigated with different levels of concentrations of recommended nutrient solutions. Three different sources of calcium such as calcium chloride ($CaCl_2$), calcium nitrate Ca $(NO_3)_2$ and calcium propionate $Ca(C_2H_5COO)_2$ in different six concentrations (0, 180, 240, 300, 360 and 420 mg L^{-1}) were added to the root zone at depth of 15 to 25 cm. The addition of calcium to the root zone was started one month after transplanting of seedlings and continued fortnightly for two months. The N, P, K, Ca and Cl content were measured from the leaves during the experiment. Similarly growth observations on stem height, stem diameter, root number, root length and average root diameter were measured one month after transplanting. The results indicate that the N content is higher under calcium nitrate and calcium propionate treatments. However application of calcium through calcium chloride decreased the N content. The potassium (P) content was not influenced under different sources of calcium instead the potassium content decreased with increasing calcium concentration. Furthermore, even though the calcium content in plant was not significantly affected by the different sources of calcium, there were significant differences between control and 300 mg L^{-1} calcium containing nutrient solution. Among the growth parameters, stem diameter and root length were maximum under the application of 240 and 180 mg L^{-1} nutrient solution, respectively. However, with the exception of control, the differences for other treatments could not reach the level of significance.

Keywords: Papaya, calcium sources, nutrient content, morphology.
Abbreviation: ANOVA_Analysis of variance; Ca_Calcium; N_Nitrogen.

Introduction

Papaya (*carica papaya* L.) belongs to the family *Caricaceae*, and is a large perennial plant with a rapid growth (Paull and Duarte, 2011). It is an important fruit in Malaysia, ranking third after durian and banana (Ali et al., 2010). The Eksotika II cultivar is a high yielding and quality F_1 hybrid released by Malaysian Agricultural Research and Development Institute (MARDI). Fruit of Eksotika II has obtained popularity in the domestic and export market (Shukur and Shokri, 1997), but the harvested fruit is prone to anthracnose infection. The most common intervening factors of the disease are using hot water, fungicides and biological materials like chitosan. Calcium is an alternative agent that uses as a firming element for fruit and to decrease decaying anthracnose disease of papaya fruit after harvesting (Eryani-Raqeeb et al., 2009). Its effectiveness for both plant growth and fruit quality in papaya can be investigated. Calcium (Ca) is a vital macronutrient element for normal plant development, taking part in copious physiological and biosynthesis processes (Barker and Pilbeam, 2007). Calcium is engaged in plant growth and development, cell-cell adherence (Marry et al., 2006). It is applicable in declining fruit decay and increasing firmness and storage (Eryani- Raqeeb et al., 2009). Thus, Ca deficiency could be led to the disturbance of metabolic activities of growing tissues and growth abnormality (Hepler, 2005). It can be used as an adage to the soil or sprayed foliar directly to increase fruit quality (White and Broadly, 2003). Calcium uptake is confined to roots tips, and takes place at greater rates in actively growing meristemic zones than in older sections (Mengel et al., 2001) delivery to the xylem is larger in the root area, where caesarian band is discontinuous (White, 2001). Calcium deficiency is most probably in organs with low transpiration rates. Translocation to them is largely by root pressure and is stimulated by formation of new cation exchange sites (Limami and Lamaze, 1991). Agronomically, calcium is needed for root and stem growth (Ganmore-Neumann and Davidov, 1993) and can enhance calcium in the foliar (Volpin and Elad, 1991). Shams et al. (2012) mentioned the advantages of calcium in various metabolic and growth processes. The present study explores the effects of root zone application of calcium on papaya under controlled environment to Eksotika II.

This study was planned and conducted to evaluate the effect of different calcium sources and concentrations on calcium content and papaya growth. Subsequently, this will be the basis to apply calcium at pre-harvest, aiming the anthracnose control of Eksotika II papaya at post-harvest.

Results

Nutrient concentration

Table 1s shows the effects of different calcium sources, concentrations and their interactions on nutrient absorption of papaya. The interaction between sources and concentrations (Fig. 1) had significant influence on N content. The effects of different calcium concentrations varied at different sources of calcium. The nitrogen (N) content found to be increased with the increase of calcium concentrations when the calcium nitrate was the source. There was an increase in nitrogen content under application of 300-360 mg L^{-1} calcium nitrate, while the calcium chloride source decreased the nitrogen content when used at the same range above (Fig. 1). However, for calcium propionate, N content had a linear trend between 180-360 mg L^{-1} and after that there was a decrement in N content. There were no significant differences among three sources and six concentrations of calcium for phosphorous content (Table 1). For potassium, highest content was observed when the source of calcium was $Ca(NO_3)_2$. In addition, it was noticed that the potassium content was decreased with increased supply of calcium. There were no significant differences amongst the three sources of calcium in term of calcium content (Table 1). However, there were significant differences between control and 300 mg L^{-1} calcium application on calcium content, and it was higher in treatment supplying 300 mg L^{-1} calcium. There were no significant differences among the concentrations of calcium in calcium chloride for chloride content in leaves (Table 2).

Effect on growth parameters

Table 3. shows the effect of Ca sources and concentrations on morphological traits of papaya. The stem height and its diameter were not significantly differed due to three sources of calcium, while the different concentrations had significant impact on growth parameters (Table 3). Stem height and diameter was significantly higher in the papaya raised on 240 -180 mg L^{-1} calcium concentrations, respectively, over control. The data showed that different concentrations of calcium had diferent efects on root growth. There was no significant effect of sources and concentrations of calcium on root number. However, different calcium concentrations had significant effect on root length i.e. control and 180 mg L^{-1}, (Table 3). For root diameter, there was no significant difference among sources, but in term of concentrations, there were significant differences between control and 420 mg L^{-1}, while 420 mg L^{-1} had the highest effects on root diameter (Table 3).

Discussion

Recent findings show that calcium has a direct and positive effect on nitrogen assimilation, which increases content of nitrogen with activating the enzymes responsible for N assimilation (Fenn et al., 1994; Lopez-Lefebre et al., 2000). In this study, the highest increased nitrogen content was caused by $Ca(NO_3)_2$ source, which has a nitrogen based compound (Swietlik, 2006). In carrot and tomato, higher level of calcium nitrate results to higher nitrogen content in the storage roots and fruits, respectively (Smolen and Sady, 2009; Peyvast et al., 2009).

Higher potassium content due to calcium nitrate may be linked to the synergic influence of potassium and nitrogen (Premaratne and Oertli, 1994). Furthermore, it was noticed that the potassium content was decreased with increased supply of calcium. This might be rooted in antagonism between calcium and potassium (Zharare et al., 2009). In the present study, the calcium content in leaves was within the ranges reported previously (Milles and Benton-Jones, 1996). Calcium is transported in xylem from root to upper parts of plant by transpiration force. It is worth mentioning though calcium content in leaves through all the sources was not significantly affected. The calcium content in leaves was enhanced by the increase of calcium concentration within nutrition. It seems that using three sources of calcium in root zone of papaya does not have any effect on calcium content in leaves. Our data confirms the findings of (Albino-Garduno et al., 2008) and (Shams et al., 2012) demonstrated that calcium content in leaves increased by calcium nitrate, and calcium chloride added to the root in gerbera and rosa in controlled conditions, respectively. Results of chlorine measurement showed at all calcium treatments, the range of chlorine concentration was between 0.60 -0.93% in leaves that was well below the toxicity threshold of papaya (Reuter and Robinson, 1997).

Results showed that increasing of calcium concentration in nutrition solution enhanced stem length and diameter of papaya seedlings. The outcomes is in agreement with Ehret et al. (2005) that calcium increase strengthened stem length and diameter in rosa (Shams et al., 2012). Calcium is necessary for cell division and elongation (Mengel et al., 2001). Cell wall stability happens with an increase in rigidity (Hirschi, 2004). The increased stem height and diameter in this research affirms the findings of Seny et al. (2011), indicated shoot height in potato was enhanced by increasing calcium concentration in the media.

Calcium levels also influenced the root development. Calcium concentrations led to thickness and elongation of root system. Hence, it can be deducted that calcium is required for root growth and total cut off of calcium in root zone will decrease root growth and gradually roots will die (Mengel et al., 2001; Poovaiah and Reddy, 1991). Also, in barley and alfalfa seedlings root growth is enhanced with the increasing calcium concentrations (Hussain et al., 2002; Grewal and Williams, 2003). Ganmore-Neumann and Davidov, (1993); and Shams et al. (2012) showed that at low calcium levels, roots were short and in high levels they were fibrous. Root elongation is heavily dependent on cell turgor pressure and stability of cell wall, and calcium is required for stability of pectin in cell wall (Shams et al., 2012).

Materials and methods

Conduct of experiment

A pot culture experiment was planned and conducted to study the effect of application of different calcium sources and concentration around the root zone on nutrient content and growth attributes of papaya during 2010-11 at Agro technology Unit, University Agriculture Park (TPU), Universiti Putra Malaysia, Serdang, Selangor. The Seeds of Papaya cultivar Eksotika II were obtained from MARDI. The seeds were germinated in 25-cells polypropylene filled with coco peat and paddy husk. Six-week-old papaya seedlings, each having more than four fully expanded leaves

Table 1. Main and interaction effects of three sources of calcium and six concentrations added to the root zone on mineral nutrients content in leaves of Eksotika II. After transplanting, Ca was added to the root zone. It is to be noted that the duration of the treatments was two months on a fortnightly base.

Factor	Parameter			
	N (%)	P (%)	K (%)	Ca (%)
Source (S)				
$CaCl_2.2H_2O$	1.99a[z]	0.36a	4.20ab	2.11a
$Ca(NO_3)_2.4H_2O$	1.71b	0.43a	4.48a	2.18a
$Ca(C_2H_5COO)_2$	1.82ab	0.36a	4.09b	2.16a
Concentrations (mg L^{-1}) (C)				
0	1.97ab	0.40a	5.06a	1.67c
180	1.53c	0.35a	4.54b	2.14abc
240	1.80c	0.36a	4.01c	2.08abc
300	1.94ab	0.37a	4.01c	2.48ab
360	2.20a	0.45a	3.98c	2.56a
420	1.59c	0.36a	3.93c	1.99bc
Interaction (S×C)	**	ns	ns	ns

[z]Means followed by the same letter in the same column are not significantly different at ($p \leq 0.05$) at DMRT.
[ns,*] Non-significant and highly significant at $p \leq 0.05$.

Table 2. Effects of six concentrations of calcium chloride added to the root zone on chloride content in leaves of Eksotika II.

Treatment	Parameter
$Cacl_2$. $2H_2O$ mg L^{-1}	Cl (%)
0	0.60a*
180	0.66a
240	0.93a
300	0.90a
360	0.76a
420	0.76a

*Means followed by the same letter in the same column are not significantly different at ($p \leq 0.05$) at DMRT

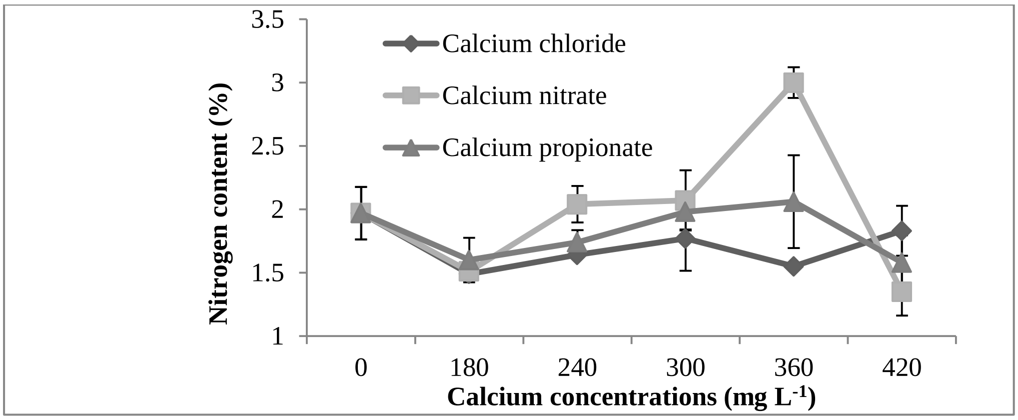

Fig 1. Interaction effect of three sources and six concentrations of calcium on nitrogen content in leaves of papaya seedlings. Vertical bars are standard error of the means

and uniform sizes were transplanted into 5L black polyethylene bags containing coco peat and paddy husk (50:50 v/v). Physico-chemical properties of media are shown in Table 4.

The seedlings were arranged in rows at 120 cm between bags and 200 cm between rows. The experiment lasted until 90 days after transplanting and the plants were placed in a net house at Agro Technology Unit, University Agriculture Park (TPU). Different levels of concentrations of nutrient solution recommended by Mardi (2009) were used to fertilize the plants. The concentration of applied macro-nutrients and micro-nutrients were as Nitrogen 0.94, P 0.32, K 0.74, Ca 1.46, Mg 1, S 1.38 (mM) Fe 3.54, Zn 1.22, Mn 0.89, Cu 1.61, B 0.18, and Mn 0.94 (µM).

The electrical conductivity (EC) and pH of the nutrient solution for total period of the experiment were maintained at 2.5 dS m^{-1} and 6, respectively. A total volume of 600 ml day^{-1} of the nutrient solution was applied to each polybag using an automatic drip irrigation system. Maximum and minimum temperatures in the shelter were 35 and 23ºC, respectively while, relative humidity was between 80-90%. The Photosynthetic active radiation (PAR) was amid 700-800µmol m^{-2} sec^{-1}. Treatments i.e. application of different levels of nutrient solutions began one month after transplanting. There were 18 treatment combinations of three sources of calcium, $CaCl_2$. $2H_2O$ (MW=147.03 g mol^{-1}), $Ca(NO_3)_2$.$4H_2O$ (MW=236.15 g mol^{-1}), and calcium propionate (MW=186.21 g mol^{-1}) and six concentrations (0, 180, 240, 300, 360 and 420 mg L^{-1}) replicated thrice in factorial experiment in Randomized Complete Block Design (RCBD). The treatments were administered every fortnightly to the root zone (1 to 25 cm deep) with other nutrient in three

Table 3. Main and interaction effects of three sources of calcium and six concentrations of calcium added to the root zone on shoot and root traits of Eksotika II. After transplanting, Ca was added to the root zone. It is to be noted that the duration of the treatments was two months in a fortnightly base.

Factor	Parameters				
	Stem height (cm)	Stem diameter (mm)	Total root number	Total root length (cm)	Average root diameter (mm)
Source (S)					
$CaCl_2.2H_2O$	138.39a*	39.89a	7015.1a	1356.8a	0.93a
$Ca(NO_3)_2.4H_2O$	135.89a	40.96a	7560.4a	1416,6a	0.87a
$Ca(C_2H_5COO)_2$	138a	39.22a	6575.6a	1471.7a	0.87a
Concentrations (mg L^{-1}) (C)					
0	115.3c	35.13b	7075a	1239.7b	0.79b
180	126.8bc	40.53a	6242.7a	1563.9a	0.92ab
240	145.5a	42.36a	7499a	1380.8ab	0.88ab
300	151.6a	41.78a	7283.8a	1484.2ab	0.90ab
360	144.6a	40.96a	7627.8a	1473ab	0.88ab
420	140.4ab	39.36ab	6074a	1348.9ab	0.96a
Interaction (S×C)	ns	ns	ns	ns	ns

*Means followed by the same letter in the same column are not significantly different at ($p \leq 0.05$) at DMRT

ns Non-significant at $p \leq 0.05$

Table 4. Physico-chemical properties of coco peat and paddy husk media.

Organic matter (%)	Bulk density (gm cm^{-3})	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
40.71	0.25	0.46	0.07	0.86	0.49	0.1

irrigation events (8:00, 10:00, and 16:00 h), and in each time 200 ml was used.

Determination of nutrient concentration from leaves

The experiment was lasted ninety days after transplanting. Measurements of plant parameters were carried out two weeks after the treatments applied i.e. 104 days after transplanting. Six fully matured leaves per treatment were harvested from the middle of the crown of each plant and then oven dried in 70°C and ground with 2 mm mesh grinder. Then, 2 mL H_2SO_4 was added to the mixture followed by 2 mL H_2O_2. Then, samples were put into a digestion flask with 280ºC for 40 minutes. Following completion of digestion, the solution was made up to a volume of 100 ml with deionised water and then analysed using the Atomic Absorption Spectrometer (AAS) (Perkin Elmer Model 3110) to determine the concentrations of potassium, and calcium in the samples. Nitrogen and phosphorus concentrations were analysed using Auto analyser (AA) (Perkin Elmer Model 403). For chloride measurement, chloride concentration was determined according to Mohr's method described by Johnson and Ulrich (1959). Grounded leaf samples (0.50 g) were extracted with 25 ml double distilled water. Then the mixture was shaken by a shaker for 30 minutes and filtered by a filter paper. The filterate (10 ml) with one drop of Potassium chromate (5%) as indicator was titrated using silver nitrate (0.1 M) to an endpoint red-brown. The results were expressed as percentage.

Measurement of growth parameters

Plant height was measured from the media line to the apical part of plant. Plant diameter was measured at three fixed locations of the plant (10 cm, 60 cm and 120 cm from the base of the plant) and the average was calculated. The roots were carefully washed and removed from the media. Root morphology measurements i.e. total root number and length and average root diameter were measured using Root Scanner Image Analyser (Model Seiko Epson Corp., Japan and Winrhizo ProV 2007 software). Dry weights of leaves were obtained by oven drying at 70ºC for 72 h.

Statistical analysis

The observations regarding nutrient concentrations and growth parameters were analysed statistically in factorial experiment in Randomized Complete Block Design (RCBD). Data were subjected to Analysis of Variance (ANOVA) using Statistical Analysis System (SAS) version 8.2 (SAS Institute Inc., Cary, NC, USA). The treatments showing significant effect were compared using Duncan Multiple Range Test (DMRT) at significant level of $p \leq 0.05$.

Conclusion

Based on results, it can be concluded that different sources and concentrations affect the mineral content differently. Also, different concentrations of calcium affect growth parameters differently. Thus, this experiment will be an introduction for the next experiment to observe the effects of different sources and concentrations of calcium as pre-harvest treatment on storage life and yield of papaya in controlled environment.

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